Journal of the American Heart Association

CONTEMPORARY REVIEW

Cardiac Graft Assessment in the Era of Machine Perfusion: Current and Future Biomarkers

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ABSTRACT: Heart transplantation remains the treatment of reference for patients experiencing end-stage heart failure; unfortunately, graft availability through conventional donation after brain death is insufficient to meet the demand. Use of extended-criteria donors or donation after circulatory death has emerged to increase organ availability; however, clinical protocols require optimization to limit or prevent damage in hearts possessing greater susceptibility to injury than conventional grafts. The emergence of cardiac ex situ machine perfusion not only facilitates the use of extended-criteria donor and donation after circulatory death hearts through the avoidance of potentially damaging ischemia during graft storage and transport, it also opens the door to multiple opportunities for more sensitive monitoring of graft quality. With this review, we aim to bring together the current knowledge of biomarkers that hold particular promise for cardiac graft evaluation to improve precision and reliability in the identification of hearts for transplantation, thereby facilitating the safe increase in graft availability. Information about the utility of potential biomarkers was categorized into 5 themes: (1) functional, (2) metabolic, (3) hormone/prohormone, (4) cellular damage/death, and (5) inflammatory markers. Several promising biomarkers are identified, and recommendations for potential improvements to current clinical protocols are provided.

Key Words: biomarkers ■ donation after circulatory death ■ ex situ heart perfusion ■ extended-criteria heart donors ■ heart transplantation

Heart transplantation is the gold standard treatment for improving survival and quality of life in patients with end-stage heart disease; however, graft availability through conventional donation after brain death (DBD) is insufficient to meet the need for all patients.[1] The number of patients awaiting cardiac transplantation has continuously increased in Europe and the United States over the past 20 years.[1,2]

Approaches to improve cardiac graft availability include the use of extended-criteria donors (ECDs) or donation after circulatory death (DCD). Although reports with ECDs and DCD are encouraging, clinical protocols have yet to be optimized. Improved methods of graft evaluation are of critical importance, not only for ensuring the best patient outcomes by correctly identifying suitable grafts and permitting the exclusion of grafts with excessive cellular dysfunction and damage,[3] but also for the identification and development of optimized clinical transplant protocols.

The organ shortage has also stimulated the development of ex situ organ perfusion systems as an alternative to conventional static, cold storage that can help to improve cardiac graft quality and availability, especially in situations in which grafts may be particularly susceptible to ischemic injury, such as those from ECDs or DCD, or when long transport times cannot be avoided. The Organ Care System Heart (OCS) by Transmedics has been developed for continuous, normothermic graft perfusion, and several systems, such as the Steen Heart Preservation System or the

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For Sources of Funding and Disclosures, see page 27.

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Nonstandard Abbreviations and Acronyms

DAMP	damage-associated molecular pattern
DBD	donation after brain death
DCD	donation after circulatory death
ECD	extended-criteria donor
HEP	high-energy phosphate
H-FABP	heart-type fatty acid binding protein
HSP	heat shock protein
MP	machine perfusion
OCS	Organ Care System Heart

HeartPort System, are currently in development/clinical testing for hypothermic graft perfusion.[4] During ex situ, machine perfusion (MP) with the OCS, the graft is maintained in a beating, unloaded state.[3] To evaluate graft quality, variables, such as heart rate, rhythm, aortic pressure, coronary flow, and lactate profiles, are monitored during organ perfusion.[3,5] For standard-criteria donor hearts preserved with the OCS or conventional cold-static storage, 30-day recipient and graft survival is similar, as is the incidence of cardiac allograft vasculopathy,[6] demonstrating the short-term safety of this approach.[7] Furthermore, with organs previously not considered suitable for transplantation and/or higher-risk recipients, MP is associated with excellent short-term outcomes.[8] In DCD heart transplantations requiring graft transport between centers, the OCS has been used, and patient outcomes are similar to those with conventional DBD at 1- and 4-year time points.[3,9,10] Thus, although still in its early stages, normothermic perfusion storage with the OCS appears promising.

To optimize patient outcomes from all donor pools, graft evaluation procedures must also evolve. Indeed, transplant criteria for conventional, DBD grafts are used; however, hearts meeting these criteria are not necessarily protected from rejection or cardiac allograft vasculopathy. Furthermore, increased use of ECDs or DCD may increase the risk of transplanting unsuitable donor hearts and lead to early graft failure. With DBD, in addition to donor inclusion/exclusion criteria, cardiac graft selection relies heavily on donor monitoring, and involves consideration of parameters such as blood pressure, electrocardiographic changes, periods of hypotension and/or cardiopulmonary resuscitation, drug history, history of hypertension, and the need for inotropic support. Up to two-thirds of potential donor hearts are rejected before retrieval on the basis of the above criteria; however, none of these criteria alone precludes successful transplantation.[11] Furthermore, up to 50% of retrieved grafts are rejected because of heart malfunction detected only after detailed inspection.[11] Thus, on one hand, there is a pool of donor organs that is rejected because their posttransplantation function cannot be predicted with confidence, whereas on the other hand, resources are wasted in the procurement of nonsuitable organs.[11] It is therefore critical to optimize evaluation strategies to more effectively select for grafts in which adequate quality is achievable.[11,12]

Although cardiac grafts from different types of donors are subjected to varying conditions before procurement, multiple markers of damage are likely to be relevant regardless of donor type. For example, DCD grafts are expected to undergo warm ischemia between circulatory arrest and procurement; however, ischemic damage may also occur during cardioplegia with DBD grafts, particularly with older donors.[12] Furthermore, in both DCD and DBD donors, a catecholamine surge occurs before graft procurement. This "adrenergic storm" can induce peripheral vasoconstriction and subsequently lead to transient ischemia of organs.[13]

Taken together, improvement of clinical protocols to evaluate donor hearts and predict posttransplant function may not only provide better clinical outcomes, but could aid in expanding the donor pool and decreasing the number of patients awaiting a suitable graft. A greater consideration of cardiac biomarkers is of particular interest in light of recently available MP technologies that enable monitoring of multiple parameters over time during graft storage and transport. With this review, we aim to summarize the current knowledge of biomarkers that hold particular promise for cardiac graft evaluation to improve our precision and reliability in the identification of hearts for transplantation, thereby facilitating a safe increase in graft availability via expansion of the donor pool.

METHODS

A systematic literature search of the PubMed database was performed with terms: "(heart or cardiac) AND (transplant or transplantation) AND biomarker AND (graft evaluation or rejection) NOT kidney NOT liver NOT lung NOT pancreas NOT (islet or islets) NOT bowel NOT pediatric NOT stem cell." The search was limited to English. A total of 1082 (updated July 24, 2020) articles were retrieved. All abstracts were reviewed to exclude irrelevant publications.

All retained articles underwent in-depth review and were assigned to categories of biomarkers according to the following indicators: (1) function, (2) metabolism, (3) hormone/prohormone, (4) cellular damage/death, and (5) inflammation. Additional PubMed searches

were performed in each specific area to ensure that no relevant articles were overlooked. Cited references in all retained publications were carefully examined, and relevant publications were reviewed in detail.

Given that interventions to evaluate graft quality before heart procurement may not be permitted/possible with all donors, and that both DBD and DCD hearts are exposed to catecholamine storms that are potentially damaging to cardiac grafts, we have focused our attention on biomarkers monitored from the time of procurement until transplantation. Aspects considered particularly relevant for cardiac biomarkers are summarized in the tables. These include study model and design, details of biomarker sampling, outcomes, and predictive value. Predictive value was indicated as "yes" when statistically significant evidence between the measured predictor and outcome was provided (eg, statistically significant correlation). Predictive value was indicated as "no" when evidence (eg, correlation or receiver operating characteristic analysis) was provided, but no statistically significant relationship was observed. In several cases, indirect data were considered to either "support" or "not support" a predictive value, as indicated in tables.

RESULTS AND DISCUSSION

The introduction of MP technologies holds enormous potential for optimizing graft evaluation strategies in heart transplantation. Given that cardiac MP is still in its infancy, a limited number of studies investigating graft evaluation during MP were retrieved with our literature searches. Nonetheless, evidence supporting the utility of various biomarkers measured between procurement and transplantation has been reported and is summarized in Figure 1 and in the following sections, where also suggestions for possible future strategies are presented.

Functional Markers

Contractile Function

Unlike DBD, antemortem functional graft evaluation is generally not performed in DCD, but rather during MP or normothermic regional perfusion (Figure 2). During MP, contractile function is evaluated by visual inspection. Criteria for transplantation acceptance for contractile function of the loaded DCD heart during normothermic regional perfusion are as follows: cardiac index >2.5 L/min per m^2, central venous pressure <12 mm Hg, pulmonary capillary wedge pressure

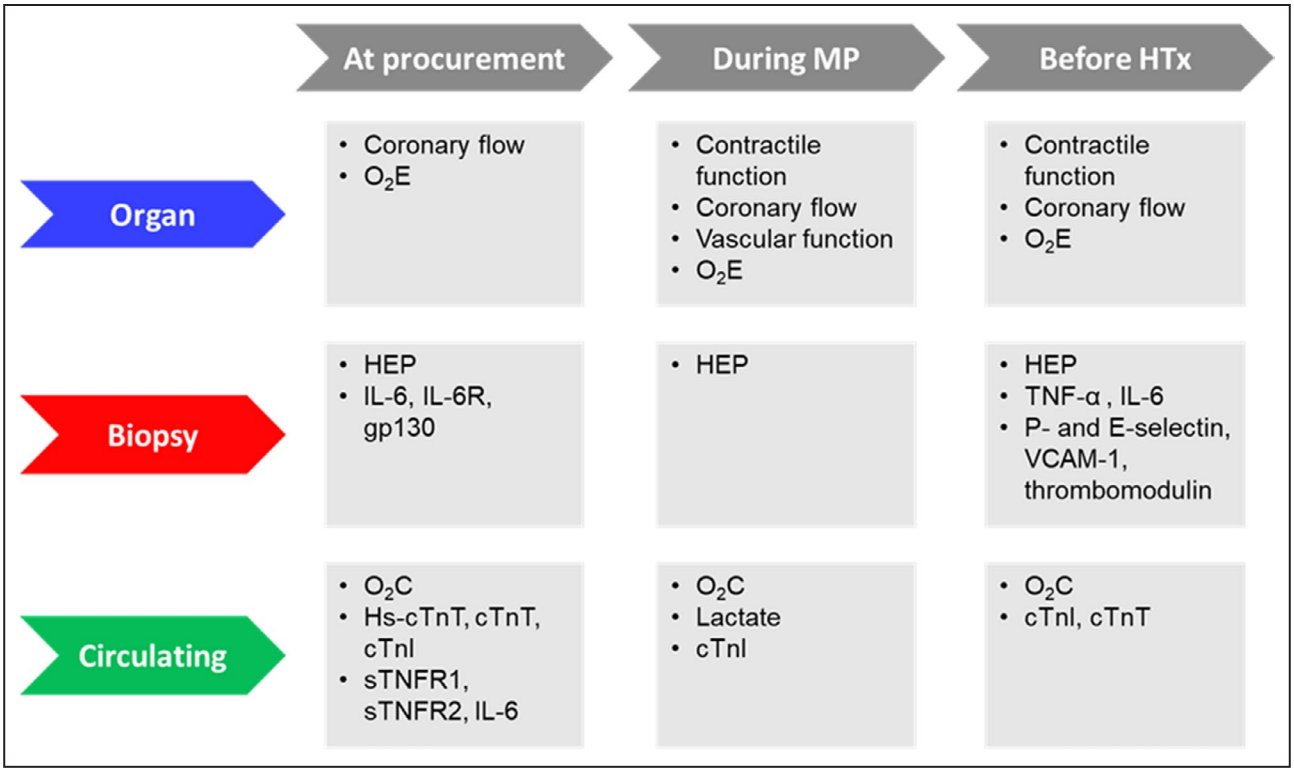

Figure 1. Biomarkers with reported potential value when evaluated during graft management.
cTnI indicates cardiac troponin I; cTnT, cardiac troponin T; gp130, glycoprotein 130; HEP, high-energy phosphates; Hs-cTnT, high-sensitivity cTnT; HTx, heart transplantation; IL-6, interleukin 6; IL-6R, IL-6 receptor; MP, machine perfusion; O_2C, cardiac oxygen consumption; O_2E, cardiac oxygen efficiency; sTNFR, soluble tumor necrosis factor receptor; TNF-α, tumor necrosis factor-α; and VCAM-1, vascular cell adhesion molecule 1.

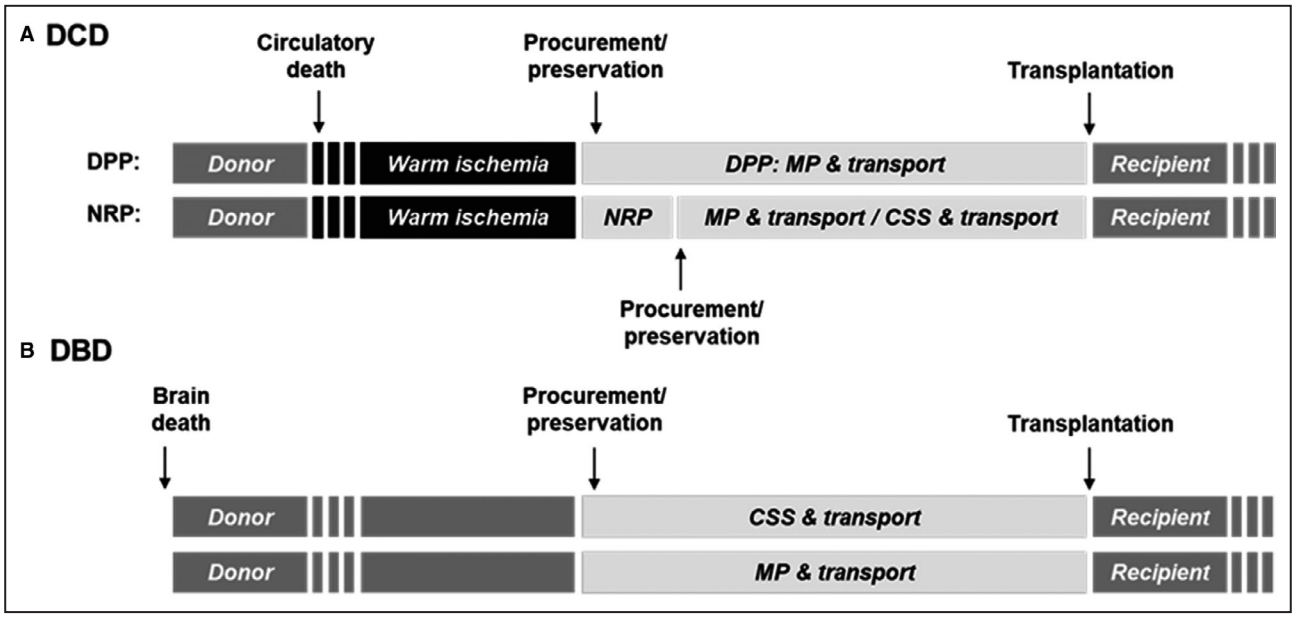

Figure 2. Schematic protocols for conditions before and during heart procurement and storage.
A, Hearts obtained with donation after circulatory death (DCD) are subjected to a period of warm, global ischemia, before procurement. In the direct procurement and perfusion protocol (DPP), grafts are stored and transported using normothermic machine perfusion (MP). In the normothermic, regional perfusion (NRP) protocol, the heart is reperfused in situ. Only after functional evaluation is it procured, stored, and transported using normothermic MP or cold, static storage (CSS). **B**, Hearts obtained with donation after brain death (DBD) remain perfused until organ procurement. Hearts are then stored and transported using CSS or normothermic MP.

<12 mm Hg, and left ventricular ejection fraction >50% on transesophageal echocardiography in human DCD transplantation.[14]

Studies in both porcine and rodent DCD models support the concept that contractile evaluation during MP is of aid in graft evaluation (Table 1[15–19]). White and colleagues reported that several contractile parameters predict simultaneously measured myocardial performance (cardiac output×heart weight^{-1}) in a porcine model, whereas end-systolic volume and end-systolic pressure-volume relationship were not helpful.[16] In line with this, Ribeiro and colleagues described that invasive and noninvasive measures of left ventricle contractility strongly correlated with cardiac function following transplantation.[15] Interestingly, several parameters measured during ex vivo, unloaded perfusions in rat DCD models have been reported to correlate with cardiac functional recovery on reloading.[17–19]

Notably, functional assessments of DCD grafts in clinical practice provided better correlations with myocardial performance than metabolic variables during MP in several studies.[9,14,20] Although the supremacy of functional parameters highlights the need for an MP device capable of assessing the donor heart in a physiologic, loaded mode,[16] functional assessment has not been evaluated in unloaded human hearts. It may be that functional evaluations performed in unloaded hearts also provide valuable information, which would be highly advantageous given that this type of preparation is technically much less demanding.

Vascular and Endothelial Function

Coronary vascular dysfunction is common in DBD heart recipients. Notably, the index of microvascular resistance, assessed early after heart transplantation, predicts death or retransplantation.[21] Endothelial dysfunction is an early marker for intimal thickening and graft atherosclerosis,[22] and changes in coronary endothelial function predict progression of allograft vasculopathy after transplantation.[23] Correspondingly, endothelial preservation helps to delay allograft vasculopathy.[24] As endothelial cells are more susceptible to ischemia-reperfusion injury than cardiac myocytes, endothelial dysfunction may be present even before graft procurement, particularly in DCD hearts.[25]

Preclinical studies indicate that various measures of vascular function correlate with heart recovery in ex situ preparations (Table 2[15,17–19,25–28]). Coronary flow and the ability of the vasculature to adapt coronary flow in response to ischemia and reperfusion (hyperemic response; coronary flow reserve) are indicators of vascular function. In preclinical models, coronary flow in loaded preparations or coronary flow and time to peak coronary flow in unloaded preparations consistently correlate with functional outcomes. These findings support the concept that assessment of vascular

Table 1. Contractile Function

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value
Multiple contractile function measures[15]	DCD, preclinical (pig)	Parallel, 2-arm study, unloaded and loaded MP at 37°C and orthotopic HTx: 1. Normal (non-DCD, non-DBD) hearts with median WIT of 2 min (n=9) 2. DCD with median WIT of 25 min (n=8)	At start of loaded MP (after 3 h unloaded MP)	Left ventricular function:	
				Stroke work at 3 h posttransplant	Yes: DP, dP/dt max, PRSW, NI E_{max}, NI PRSW (ρ=Pve, P<0.05 for all), dP/dt min, τ, EDPV relationship (ρ=Nve, P<0.05 for all)
				PRSW at 3 h posttransplant	Yes: dP/dt max, PRSW, NI E_{max} (ρ=Pve, P<0.05 for all), τ, EDPV relationship (ρ=Nve, P<0.05 for both)
					No: DP, dP/dt min, NI PRSW (P=NS for all)
				Cardiac index at 3 h posttransplant	Yes: DP, dP/dt max, PRSW, NI E_{max}, NI PRSW (ρ=Pve, P<0.05 for all)
					No: dP/dt min, τ, EDPV relationship (P=NS for all)
				Right ventricular function:	
				RVSWI at 3 h posttransplant	Yes: Stroke work (ρ=Pve, P<0.05)
					No: DP, EDPV relationship, NI PRSW (P=NS for all)
				Cardiac index at 3 h posttransplant	Yes: NI PRSW (ρ=Pve, P<0.05)
					No: DP, stroke volume, EDPV relationship (P=NS for all)
Multiple contractile function measures[16]	DCD, preclinical (pig)	Parallel, 2-arm study, loaded MP at 37°C for: 1. Normal (non-DCD, non-DBD) hearts with mean WIT of 4.6±0.2 min (n=9) 2. DCD hearts with mean WIT of 27.6±0.3 min (n=37)	At start of loaded MP	Cardiac index (cardiac output per heart weight) at start of loaded MP (simultaneous with biomarker)	Yes: DP (R^2=0.569, P<0.001) dP/dt max (R^2=0.537, P<0.001) dP/dt min (R^2=0.74, P<0.001) dV/dt max (R^2=0.616, P<0.001) dV/dt min (R^2=0.321, P<0.001) EDP (R^2=0.202, P<0.002) EDPV relationship (R^2=0.143, P<0.05) ejection fraction (R^2=0.80, P<0.001) ESP (R^2=0.512, P<0.001) stroke work (R^2=0.76, P<0.001) τ (R^2=0.51, P<0.001)
					No: EDV (R^2=0.004, P=NS) ESPV relationship (R^2=0.012, P=NS) ESV (R^2=0.081, P=NS)

(*Continued*)

Table 1. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value
Multiple contractile function measures[17]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=7–8 per group)	At 10 min MP	LV work at 60 min MP	Yes: LV work, DP, heart rate, dP/dt max (ρ=Pve, P<0.01 for all), dP/dt min (ρ=Nve, P<0.01)
				TP at 60 min MP	Yes: DP, dP/dt max, heart rate, LV work (ρ=Pve, P<0.01 for all)dP/dt min (ρ=Nve, P<0.01)
				CO at 60 min MP	Yes: DP, dP/dt max, heart rate, LV work (ρ=Pve, P<0.01 for all) dP/dt min (ρ=Nve, P<0.01)
				dP/dt max at 60 min MP	Yes: DP, dP/dt max, heart rate, LV work (ρ=Pve, P<0.01 for all) dP/dt min (ρ=Nve, P<0.01)
				dP/dt min at 60 min MP	Yes: DP, dP/dt max, heart rate, LV work (ρ=Nve, P<0.01 for all) dP/dt min (ρ=Pve, P<0.01)
Multiple contractile function measures[18]	DCD, preclinical (rat)	Parallel, 3-arm study in hearts subjected to WIT, as described below, followed by 10 min procurement reperfusion, cardioplegic flush, CSS for 3 h, 120 min loaded, normothermic MP: 1. 15 min WIT (n=6) 2. 20 min WIT (n=6) 3. 25 min WIT (n=5)	(A) At 10 min procurement reperfusion (before CSS) (B) At 10 min loaded, normothermic MP (after CSS)	CO at 120 min loaded, normothermic MP	Yes: DP, dP/dt min, dP/dt max, heart rate, LV work (A and B; ρ=NR, P<0.05 for all)
					No: EDP, P_{min}, PSP (A and B; P=NS for all)
				PSP at 120 min loaded, normothermic MP	Yes: DP, dP/dt max, dP/dt min, heart rate, LV work (A and B; ρ=NR, P<0.05 for all) PSP (B only; ρ=NR, P<0.05)
					No: EDP, P_{min} (A and B; P=NS for both)
				DP at 120 min loaded, normothermic MP	Yes: DP, dP/dt max, dP/dt min, heart rate, LV work (A and B; ρ=NR, P<0.05 for all) PSP (B only; ρ=NR, P<0.05)
					No: EDP, P_{min} (A and B; P=NS)
				HR at 120 min loaded, normothermic MP	Yes: HR (B only; ρ=NR, P<0.05) LV work (A and B; ρ=NR, P<0.05)
					No: EDP, DP, dP/dt max, dP/dt min, P_{min}, PSP (A and B; P=NS for all)
				dP/dt max/min at 120 min loaded, normothermic MP	Yes: DP, dP/dt max, dP/dt min, heart rate, LV work (A and B; ρ=NR, P<0.05 for all) PSP (B only; ρ=NR, P<0.05)
					No: EDP, P_{min} (A and B; P=N for both)
				LV work at 120 min loaded, normothermic MP	Yes: DP, dP/dt max, dP/dt min, heart rate, LV work (A and B; ρ=NR, P<0.05 for all) PSP (B only; ρ=NR, P<0.05)
					No: EDP, P_{min} (A and B; P=NS for both)

(*Continued*)

Table 1. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value
Multiple contractile function measures[19]	DCD, preclinical (rat)	Parallel, 4-arm study, MP (20 min unloaded+40 min loaded) at 37°C of hearts subjected to WIT of: 1. 30 min, 32°C (n=6) 2. 50 min, 32°C (n=5) 3. 55 min, 32°C (n=15) 4. 60 min, 32°C (n=5)	At 10 min MP	HR at 60 min MP	Yes: DP, dP/dt min, EDP, P_{min}, dP/dt max (ρ=NR, *P*<0.001 for all) HR (ρ=NR, *P*<0.05) LV work (ρ=NR, *P*<0.001)
					No: PSP (*P*=NS)
				PSP at 60 min MP	Yes: DP, dP/dt min, EDP, Pmin (ρ=NR, P<0.01 for all) dP/dt max, LV work (ρ=NR, *P*<0.001 for both) HR (ρ=NR, *P*<0.05)
					No: PSP (*P*=NS)
				DP at 60 min MP	Yes: DP, HR (ρ=NR, *P*<0.05 for both) dP/dt min, EDP, P_{min} (ρ=NR, *P*<0.01 for all) dP/dt max, LV work (ρ=NR, *P*<0.001 for both)
					No: PSP (*P*=NS)
				dP/dt max at 60 min MP	Yes: DP, EDP, HR, P_{min} (ρ=NR, *P*<0.01 for all) dP/dt max, dP/dt min, LV work (ρ=NR, *P*<0.001 for all)
					No: PSP (*P*=NS)
				dP/dt min at 60 min MP	Yes: DP, EDP, HR (ρ=NR, *P*<0.01 for all) LV work, dP/dt max, dP/dt min (ρ=NR, *P*<0.001 for all) P_{min} (ρ=NR, *P*<0.05)
					No: PSP (*P*=NS)
				LV work at 60 min MP	Yes: DP, dP/dt max, dP/dt min, EDP, LV work, P_{min} (ρ=NR, *P*<0.001 for all) HR (ρ=NR, *P*<0.01)
					No: PSP (*P*=NS)
				CO at 60 min MP	No: DP, dP/dt max, dP/dt min, EDP, heart rate, LV work, P_{min}, PSP (*P*=NS for all)

ρ indicates Spearman ρ; CO, cardiac output; CSS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; DP, developed pressure; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; dV/dt max, maximal first derivative of left ventricular volume; dV/dt min, minimal first derivative of left ventricular volume; EDP, end-diastolic pressure; EDPV, end-diastolic pressure-volume; EDV, end-diastolic volume; ESP, end-systolic pressure; ESPV, end-systolic pressure-volume; ESV, end-systolic volume; HR, heart rate; HTx, heart transplantation; LV work, left ventricular work (heart rate×DP); MP, machine perfusion; NI E_{max}, noninvasive maximum elastance; NI PRSW, noninvasive preload recruitable stroke work; NR, not reported; NS, not significant; Nve, negative correlation; P_{min}, left ventricular minimal pressure; PRSW, preload recruitable stroke work; PSP, peak systolic pressure; Pve, positive correlation; R^2, correlation coefficent for linear regression; RVSWI, right ventricular stroke work index; T, Tau; TP, triple product (HR–DP–dP/dt max product); and WIT, warm ischemia time.

Table 2. Vascular and Endothelial Function

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
Coronary vascular resistance[15]	DCD, preclinical (pig)	Parallel, 2-arm study, unloaded and loaded MP at 37°C and orthotopic HTx: 1. Normal (non-DCD, non-DBD) hearts with median WIT of 2 min (n=9) 2. DCD with median WIT of 25 min (n=8)	(A) At 30 min MP (B) At 3 h MP	RVSWI at 3 h posttransplant (A and B)	Yes: A and B (ρ=Nve, $P<0.05$)	
Coronary flow[26]	DBD, preclinical (pig)	Parallel, 3-arm study, 3 h brain death period, followed by in situ WIT of: 1. 0 min (n=6) 2. 10 min (n=6) 3. 20 min (n=6) All hearts then subjected to cold cardioplegic flush for 3 min, followed by HTx (120 min follow-up)	At time of cardioplegic flush	Post-HTx function (details NR)	Yes (r=NR, P=NR)	NR
				Energetic index after cardioplegic flush	Yes (R=Pve, $P<0.001$)	
Coronary flow[27]	DCD, preclinical (pig)	Parallel, 5-arm study with in situ WIT of: 1. 0 min (n=6) 2. 10 min (n=6) 3. 20 min (n=6) 4. 30 min (n=6) 5. 60 min (n=6) All hearts then subjected to cold cardioplegic flush for 3 min, followed by 2 h CSS and subsequent MP for 30 min	At time of cardioplegic flush	LVDP during MP	Yes (R=0.9, P<0.001)	NR
				Energetic index after cardioplegic flush	Yes (R=0.84, $P<0.001$)	
Coronary flow[25]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=7 or 8 per group)	At 3 min MP	CO, DP, LV work, TP at 60 min MP	Yes (ρ=Pve, $P<0.001$ for all)	Yes: O_2C (ρ=Pve, $P<0.001$); LDH (ρ=Nve, $P<0.001$); peroxynitrite (100 kDa; ρ=Nve, $P<0.05$); WIT (ρ=Nve, $P<0.001$)
				dP/dt max at 60 min MP	Yes (ρ=Pve, $P<0.05$)	
				dP/dt min at 60 min MP	Yes (ρ=Nve, $P<0.05$)	

(*Continued*)

Table 2. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
Coronary flow[17]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=7–8 per group)	At 10 min MP	CO, dP/dt max, LV work, TP at 60 min MP	Yes (ρ=Pve, P<0.01)	NR
				dP/dt min at 60 min MP	Yes (ρ=Nve, P<0.01)	
Coronary flow[18]	DCD, preclinical (rat)	Parallel, 3-arm study in hearts subjected to WIT, as described below, followed by 10 min procurement reperfusion, cardioplegic flush, CSS for 3 h 120 min loaded, normothermic MP: 1. 15 min WIT (n=6) 2. 20 min WIT (n=6) 3. 25 min WIT (n=5)	(A) At 10 min procurement reperfusion (before CSS) (B) At 10 min loaded, normothermic MP (after CSS)	CO, DP, dP/dt max, dP/dt min, LV work at 120 min loaded, normothermic MP	Yes: A and B (ρ=NR, P<0.05 for all)	NR
				HR at 120 min loaded, normothermic MP	Yes: B only (ρ=NR, P<0.05)	
Coronary flow[19]	DCD, preclinical (rat)	Parallel, 4-arm study, MP (20 min unloaded+40 min loaded) at 37°C of hearts subjected to WIT of: 1. 30 min, 32°C (n=6) 2. 50 min, 32°C (n=5) 3. 55 min, 32°C (n=15) 4. 60 min, 32°C (n=5)	At 10 min MP	DP, dP/dt max, LV work, PSP at 60 min MP	Yes (ρ=NR, P<0.05 for all)	NR
				dP/dt min, CO, HR at 60 min MP	No (P=NS for all)	
Coronary flow (time to peak)[28]	DCD, preclinical (rat)	Ex vivo WIT of 5–43 min, followed by 40 min MP (n=NR)	During MP	Power output recovery at 10 min MP	Yes (R=−0.86, P=0.005)	NR
Edema[25]	DCD, preclinical (rat)	Parallel, 5-arm study, 60 min MP in hearts subjected to WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=5–8 per group)	At 60 min MP	CO, DP, LV work, TP at 60 min MP (simultaneous with biomarker)	Yes (ρ=Nve, P<0.05)	Yes: WIT (ρ=Pve, P<0.05); LDH (ρ=Pve, P<0.05); - peroxynitrite (60 kDa; ρ=Pve, P<0.05)
				dP/dt min at 60 min MP (simultaneous with biomarker)	Yes (ρ=Pve, P<0.05)	
				dP/dt max at 60 min MP (simultaneous with biomarker)	No (P=NS)	

(*Continued*)

Table 2. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
Peroxynitrite tissue levels[25]	DCD, preclinical (rat)	Parallel, 5-arm study, 60 min MP in hearts subjected to WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=4–6 per group)	Peroxynitrite (100 kDa) at 60 min MP	CO, LV work at 60 min MP (simultaneous with biomarker)	Yes (ρ=Nve, $P<0.05$ for both)	Yes: WIT (ρ=Pve, $P<0.001$); LDH (ρ=Pve, $P<0.05$); peroxynitrite (75 kDa; ρ=Pve, $P<0.05$); peroxynitrite (60 kDa; ρ=Pve, $P<0.001$)
				DP, dP/dt max, dP/dt min, TP at 60 min MP (simultaneous with biomarker)	No (P=NS)	
			Peroxynitrite (60 kDa) at 60 min MP	DP, LV work at 60 min MP (simultaneous with biomarker)	Yes (ρ=Nve, $P<0.05$)	Yes: WIT (ρ=Pve, $P<0.05$); LDH (ρ=Pve, $P<0.05$); O_2E (ρ=Nve, $P<0.05$); edema (ρ=Pve, $P<0.05$); peroxynitrite (100 kDa; ρ=Pve, $P<0.001$)
				CO, dP/dt max, dP/dt min, TP at 60 min MP (simultaneous with biomarker)	No (P=NS)	
Vascular function[25]	DCD, preclinical (rat)	Parallel, 3-arm study, 30 min MP in hearts subjected to WIT of: 1. 21 min 2. 24 min 3. 27 min (n=6 per group)	Endothelial-dependent vasodilation (bradykinin,10^{-8} mol/L) at 30 min MP	Surrogates (DP, dP/dt max, CO, TP at 20 min MP)*	Yes (ρ=Pve, $P<0.05$ for all)	Yes: WIT (ρ=Nve, $P<0.05$)
				Surrogates (dP/dt min at 20 min MP)*	Yes (ρ=Nve, $P<0.001$)	
				Surrogates (LV work at 20 min MP)*	No (P=NS)	
			Endothelial-independent vasodilati (3×10^{-5} mol/L SNP) at 30 min MP	Surrogates (dP/dt min at 20 min MP)*	Yes (ρ=Nve, $P<0.05$)	Yes: WIT (ρ=Nve, $P<0.05$)
				Surrogates (CO, DP, dP/dt max, LV work, TP at 20 min MP)*	No (P=NS for all)	

Energetic index calculated as (ATP+0.5×ADP)/(ATP+ADP+AMP). Power output recovery expressed as ratio of reperfusion value/preischemic value for aortic flow rate×afterload pressure. ρ indicates Spearman ρ; CO, cardiac output; CSS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; DP, developed pressure; HR, heart rate; HTx, heart transplantation; LDH, lactate dehydrogenase; LV work, left ventricular work (heart rate×DP); LVDP, left ventricular developed pressure; MP, machine perfusion; NR, not reported; NS, not significant; Nve, negative correlation; O_2C, oxygen consumption; O_2E, oxygen efficiency; PSP, peak systolic pressure; Pve, positive correlation; *R*, Pearson correlation; RVSWI, right ventricular stroke work index; SNP, sodium nitroprusside; TP, triple product (HR–DP–dP/dt max product); and WIT, warm ischemia time.

*Surrogates (measured at 20 minutes MP) as indicators of LV work at 60 minutes MP.

and/or endothelial function in both DCD and DBD grafts before transplantation may be particularly promising for graft evaluation.

Although vascular function is currently monitored during MP, its predictive value before transplantation remains to be determined.[29] One advantage of coronary flow is that continuous measurements can easily be obtained during MP. However, coronary flow and vascular responses are substantially influenced by many factors, such as vasodilatory agents (eg, adenosine), cardiac oxygen demand and perfusate oxygen levels, temperature, and pressure. Thus, within individual studies, when procurement and reperfusion conditions are maintained, coronary flow may correlate well with functional recovery; however, when these conditions vary, as would be expected in clinical practice, it may be less reliable. As such, these parameters must be interpreted in light of specific perfusion conditions and may be best used in combination with other predictive indicators to offset potential disadvantages related to their susceptibility to alteration by independent factors.

Metabolic Markers

Cardiac Oxygen Consumption

Cardiac oxygen consumption during MP, in both loaded and unloaded conditions, correlates with subsequent cardiac function in a small number of preclinical DCD studies (Table 3[15–18]). Similar to coronary flow, oxygen consumption is easily measurable during MP in venous and arterial perfusate samples using a standard blood gas analyzer, but is subject to perturbations by multiple factors, including cardiac function and coronary flow. One option to improve the reliability of oxygen consumption is to consider it in combination with contractile function (eg, cardiac oxygen efficiency), which correlated well with cardiac recovery when measured in unloaded preparations in a preclinical DCD model.[17]

Lactate

The use of lactate profiles during MP for graft evaluation has yielded varying results, but may be of greater utility in DBD compared with DCD grafts (Table 4[15–20,30–32]). Lactate extraction and/or perfusate lactate changes over time are currently used as metabolic markers for graft quality in heart transplantation during MP.[7,8] Inclusion criteria ([1] net lactate extraction, [2] decreasing or stable perfusate lactate levels, and [3] perfusate lactate concentration <5 mmol/L at end MP) are based on experience with DBD hearts.[7] End-MP lactate concentration was defined in DBD hearts as a predictor of 30-day graft failure, with a sensitivity of 0.625 and a specificity of 0.975.[31] Although these criteria have been implemented to help identify suitable human[5] and porcine[33,34] DCD grafts for transplantation, a lack of sensitivity for lactate in DCD cardiac graft evaluation has been reported.[14,20] Indeed, at least 5 of 9 DCD hearts with end-MP lactate concentrations >5 mmol/L could be transplanted without compromising outcomes.[9,14,20] This lack of sensitivity may result from the fact that several factors can affect lactate profiles, including donor lactate levels, concentrations of other perfusate energy substrates, and erythrocyte metabolic rates. Lactate may thus be best used in combination with other biomarkers, and specific criteria/thresholds remain to be established for DCD graft evaluation. Interestingly, it has recently been reported in a preclinical study of mixed DCD and DBD donors that glucose profiles may be of greater value in predicting posttransplant heart function than those of lactate.[35]

High-Energy Phosphate Metabolites

Higher levels of cardiac high-energy phosphates (HEPs), whether measured at the start, during, or after the graft storage period, are associated with better graft outcomes in both clinical and preclinical studies in DBD, as well as in preclinical DCD reports (Table 5[32,36–43]). Furthermore, preclinical reports demonstrate that partial or full replacement of conventional, cold, static graft storage with continuous perfusion provides better preservation of HEPs and enhances contractile function or recovery of function in rat,[41–43] dog,[39] and pig[32,38] models. Although fewer studies have been performed with human grafts, continuous perfusion also better preserves HEPs.[44] These findings are in agreement with the concept that better preservation of metabolic homeostasis, rather than simply limiting metabolic activity, is a superior strategy for optimal graft preservation.[45]

HEPs are of particular interest as they can be rapidly quantified during MP by phosphorous-31 magnetic resonance spectroscopy in graft biopsies, albeit this technology may be limited to research centers. However, any individual measurement provides only a snapshot of tissue HEP content, whereas changes in HEP profiles over time, which may prove particularly valuable, require multiple measurements that could be obtained with biopsies during MP. Further investigations are necessary to establish sensitive and reliable indicators of graft quality with myocardial HEP content.

Uric Acid

The end product of purine catabolism, uric acid, as a marker for various outcomes, when measured in recipients of DBD hearts, has been investigated in only a few studies.[46–48] Higher posttransplant uric acid levels are associated with increased risk of developing cardiac

Table 3. Cardiac Oxygen Consumption

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
O_2C[15]	DCD, preclinical (pig)	Parallel, 2-arm study, unloaded and loaded MP at 37°C and orthotopic HTx: 1. Normal (non-DCD, non-DBD) hearts with median WIT of 2 min (n=9) 2. DCD with median WIT of 25 min (n=8)	At 3 h MP	RVSWI at 3 h posttransplant	Yes: (ρ=Pve, P<0.05)	
O_2C[16]	DCD, preclinical (pig)	Parallel, 2-arm study, loaded MP at 37°C for: 1. Normal (non-DCD, non-DBD) hearts with mean WIT of 4.6±0.2 min (n=9) 2. DCD hearts with mean WIT of 27.6±0.3 min (n=37)	Perfusate at start of loaded MP	Cardiac index (CO per heart weight) at start of loaded MP (simultaneous with biomarker)	Yes (R^2=0.283, P<0.001)	NR
O_2C[17]	DCD, preclinical (rat)	Parallel, 3-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 27 min 3. 33 min (n=5–8 per group)	At 10 min MP	Surrogates (LV work, DP, CF, and dP/dt max at 10 min MP)*	Yes (ρ=Pve, P>0.05)	Yes for WIT (ρ=Nve, P>0.01)
				Surrogate (dP/dt min at 10 min MP)*	Yes (ρ=Nve, P>0.05)	
				Surrogate (HR at 10 min MP)	No (P=NS)	
O_2E[17]	DCD, preclinical (rat)	Parallel, 3-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 27 min 3. 33 min (n=5–8 per group)	At 10 min MP	Surrogates (LV work, DP, HR, CF, and dP/dt max at 10 min MP)*	Yes (ρ=Pve, P>0.05)	Yes for WIT (ρ=Nve, P>0.01)
				Surrogate (dP/dt min at 10 min MP)*	Yes (ρ=Nve, P>0.01)	
O_2C[18]	DCD, preclinical (rat)	Parallel, 3-arm study in hearts subjected to WIT, as described below, followed by 10 min procurement reperfusion, cardioplegic flush, CSS for 3 h, 120 min loaded, normothermic MP: 1. 15 min WIT (n=6) 2. 20 min WIT (n=6) 3. 25 min WIT (n=5)	(A) At 10 min procurement reperfusion (before CSS) (B) At 10 min loaded, normothermic MP (after CSS)	CO at 120 min loaded, normothermic MP	Yes: A and B (ρ=NR, P<0.05)	NR
				DP at 120 min loaded, normothermic MP	Yes: A (ρ=NR, P<0.05) No: B (P=NS)	
				dP/dt max/min at 120 min loaded, normothermic MP	Yes: A and B (ρ=NR, P<0.05)	
				HR at 120 min loaded, normothermic MP	No: A and B (P=NS)	
				LV work at 120 min loaded, normothermic MP	Yes: A and B (ρ=NR, P<0.05)	
				PSP at 120 min loaded, normothermic MP	Yes: A (ρ=NR, P<0.05) No: B (P=NS)	

ρ indicates Spearman ρ; CF, coronary flow; CO, cardiac output; CSS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; DP, developed pressure; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; HR, heart rate; HTx, heart transplantation; LV work, left ventricular work (heart rate×DP); MP, machine perfusion; NR, not reported; NS, not significant; Nve, negative correlation; O_2C, cardiac oxygen consumption; O_2E, cardiac oxygen efficiency; PSP, peak systolic pressure; Pve, positive correlation; R^2, correlation coefficent for linear regression; RVSWI, right ventricular stroke work index; and WIT, warm ischemia time.

*Surrogates (measured at 10 minutes MP) as indicators of LV work at 60 minutes MP.

Table 4. Lactate

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/ Predictive Value
Lactate[30]	DBD, clinical	Randomized, prospective, parallel, 2-arm study with 1 relevant arm: perfusion storage/MP (n=39)	Blood perfusate during MP	Rejection or acceptance for transplantation	Supported: ↑ end MP lactate in rejected vs transplanted groups (P<0.05)
Lactate[31]	DBD, clinical	Prospective, single-arm study, MP (n=49)	Arterial and venous blood perfusate during MP (end-MP lactate, rate of perfusate lactate change)	Posttransplant outcome (graft failure within 30 d)	Yes: end-MP lactate as explanatory parameter in logistic regression model (P=0.0044)
					Yes: rate of lactate change as explanatory parameter in logistic regression model (P=0.0065)
Lactate[20]	DCD, clinical	Single-arm study of grafts subjected to MP (WIT NR; n=21)	Blood perfusate at end MP	Posttransplant cardiac index, intra-aortic balloon pump requirement, length of stay, mortality	Not supported (no difference in outcomes for hearts with lactate >5 vs <5 mmol/L (P=NR)
Lactate[15]	DCD, preclinical (pig)	Parallel, 2-arm study, unloaded and loaded MP at 37°C and orthotopic HTx: 1. Normal (non-DCD, non-DBD) hearts with median WIT of 2 min (n=9) 2. DCD with median WIT of 25 min (n=8)	(A) At 1 h MP (B) Extraction during MP (C) At 3 h MP	RVSWI at 3 h posttransplant (A and B)	Yes: B (ρ=Nve, P<0.05)
					No: A (P=NS)
				Cardiac index at 3 h posttransplant (C)	Yes: (ρ=Nve, P<0.05)
Lactate[32]	DCD, preclinical (pig)	Parallel, 2-arm study with 8–44 min warm ischemia, followed by storage conditions described below, and 1 h unloaded MP: 1. 4 h cold CSS (n=8) 2. 4 h cold MP (n=8)	Tissue samples after 4 h storage	Myocardial function after 1 h reperfusion	Supported: In MP vs CSS: ↓ intracellular lactate (P<0.01) and ↑ heart rate (P<0.05), P max (P<0.05), dP/dt max (P<0.05), RPP (P<0.01), and contractility index (P<0.05), ↓ dP/dt min (P<0.05)
Lactate[16]	DCD, preclinical (pig)	Parallel, 2-arm study, loaded MP at 37°C for: 1. Normal (non-DCD, non-DBD) hearts with mean WIT of 4.6±0.2 min (n=9) 2. DCD hearts with mean WIT of 27.6±0.3 min (n=37)	Arterial and venous blood perfusate at start of loaded MP	Cardiac index at start of loaded MP (simultaneous with biomarker)	No (all hearts considered together): arterial lactate (R^2=0.019, P=NS), venous lactate (R^2=0.001, P=NS), venoarterial lactate difference (R^2=0.006, P=NS)
Lactate[17]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min(n=5–8 per group)	Perfusate at 10 min MP	LV work, TP, CO, dP/dt max, dP/dt min at 60 min MP	No (P=NS for all)
Lactate[18]	DCD, preclinical (rat)	Parallel, 3-arm study in hearts subjected to WIT, as described below, followed by 10 min procurement reperfusion, cardioplegic flush, CSS for 3 h, 120 min loaded, normothermic MP: 1. 15 min WIT (n=6) 2. 20 min WIT (n=6) 3. 25 min WIT (n=5)	(A) At 10 min procurement reperfusion (before CSS) (B) At 10 min loaded, normothermic MP (after CSS)	Multiple functional parameters (CF, CO, PSP, DP, heart rate, dP/dt min, dP/dt max, RPP, TP) after 120 min loaded, normothermic MP	No, for A and B (P=NS for all)

(*Continued*)

Table 4. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/ Predictive Value
Lactate[19]	DCD, preclinical (rat)	Parallel, 4-arm study, MP (20 min unloaded+40 min loaded) at 37°C of hearts subjected to WIT of: 1. 30 min, 32°C (n=6) 2. 50 min, 32°C (n=5) 3. 55 min, 32°C (n=15) 4. 60 min, 32°C (n=5)	Perfusate at 10 min MP	PSP, LV work at 60 min MP	Yes (ρ=NR, P<0.05 for both)
				DP, heart rate, dP/dt max, dP/dt min, CO at 60 min MP	No (P=NS for all)

ρ indicates Spearman ρ; CF, coronary flow; CO, cardiac output; CSS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; DP, developed pressure; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; HTx, heart transplantation; LV work, left ventricular work (heart rate×DP); MP, machine perfusion; NR, not reported; NS, not significant; Nve, negative correlation; Pmax, maximal left ventricular pressure; PSP, peak systolic pressure; R, Pearson correlation; RPP, rate-pressure product (heart rate×PSP); R^2, correlation coefficient for linear regression; RVSWI, right ventricular stroke work index; TP, triple product (heart rate−DP−dP/dt max product); and WIT, warm ischemia time.

allograft vasculopathy,[46] and elevated serum uric acid concentrations at 1 year after heart transplant are associated with an increased risk of mortality compared with patients with uric acid levels below the upper quartile cutoff.[47] However, this may be less donor/graft related and more recipient related as hyperuricemia in recipients before heart transplant is associated with more severe rejection after transplant compared with patients with lower uric acid levels.[48]

In patients with ST-segment–elevation myocardial infarction, the prognostic value of circulating uric acid has been demonstrated, which may be particularly relevant for DCD graft evaluation. In one study, mortality in patients with myocardial infarction was reported as ≈3.7-fold higher in patients with uric acid concentrations in the highest quartile compared with those with uric acid concentrations of the lowest quartile.[49] However, in patients with ST-segment–elevation myocardial infarction who underwent percutaneous coronary intervention, intensive care unit complications were more prevalent in patients with higher versus lower fasting uric acid levels, and intensive care unit mortality was not statistically different.[50]

Although the potential of uric acid shows promise, its utility when evaluated before transplantation has not yet been investigated.

Hormone/Prohormone Markers

Brain Natriuretic Peptide

Several clinical studies support a role for brain natriuretic peptide (BNP) as a circulating biomarker in DBD cardiac graft assessment. BNP is released from ventricular cardiomyocytes in response to various "stress" stimuli, such as mechanical stretch, neuroendocrine activation, and tachycardia. In the context of DBD, donor plasma levels of both BNP and its precursor NT-proBNP (N-terminal pro-B-type natriuretic peptide), when measured around the time of brain death, are associated with cardiac function at the time of organ procurement and posttransplantation[51]. Furthermore, the accuracy of predicting heart function in DBD donors can be improved by combining simultaneous measurements with donor cardiac troponins.[51] In addition, as BNP and NT-proBNP are released in acute ischemia, these molecules may be of particular utility in DCD.[52] To our knowledge, the value of BNP or NT-proBNP as a biomarker of graft quality, when measured at the time of, or following, heart procurement has not been investigated.

Procalcitonin

Procalcitonin is a precursor of the hormone calcitonin and clinically used as a circulating marker of inflammation. To date, 3 small, cohort studies in DBD

Table 5. HEP Metabolites

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/ Predictive Value
MRS score (PCr/Pi+pH−7)[36]	DBD, clinical	Retrospective, parallel, 3-arm study	Graft during static storage (MRS)	Heart allocation to 1. Successfully transplanted hearts (n=14) 2. Transplanted hearts with EGF (n=3) 3. Grafts not suitable for transplantation (n=9)	Supported: MRS score progressively decreased from group 1 to group 2 and group 3 (differences among groups statistically significant; *P*=0.0001)
PCr/ATP ratio[37]	DBD, clinical	Prospective, single-arm study (n=25)	Graft during static storage (MRS)	Cardiac index 1 wk after HTx	Yes (r=0.45, *P*=0.02)
ATP[38]	DBD, preclinical (pig)	Parallel, 4-arm study: 1. 8 h CSS (n=6) 2. 8 h reperfusion storage (32°C–34°C; n=6) 3. 8 h CSS followed by orthotopic HTx (n=12) 4. 8 h reperfusion storage (32°C–34°C) followed by orthotopic HTx (n=12)	Tissue samples after 8 h storage	Myocardial function 2 h after HTx	Supported: ↑ ATP in group 2 vs 1 (*P*<0.05) with ↑ LVSP and cardiac index (*P*=0.00 for all) and ↑ cardiac output (*P*=0.001) in group 4 vs 3
ATP[39]	DBD, preclinical (dog)	Parallel, 2-arm study with graft storage conditions, as described below, followed by 1 h unloaded, normothermic MP: 1. 6 h CSS (n=9) 2. 6 h reperfusion (25°C for first 30 min, 4°C–6°C for the remaining time; n=9)	Tissue samples after 6 h storage	dP/dt max after 1 h normothermic MP	Yes (*R*=0.41, *P*=0.049)
				ATP after 1 h normothermic MP	Not supported: unchanged ATP (biomarker) after 6 h storage between groups, but ↑ ATP (outcome; *P*=0.003) in group 2 vs 1
ATP/Pi and PCr/Pi ratios[40]	DBD, preclinical (dog)	Parallel, 4-arm study: 1. 24 h CSS (n=6) 2. 24 h cold reperfusion (n=6) 3. 24 h CSS followed by HTx (n=6) 4. 24 h cold reperfusion followed by HTx (n=6)	Tissue samples after 24 h storage (MRS)	Myocardial function after HTx	Not supported: ↑ ATP/Pi and PCr/Pi ratios (*P*<0.05 for both) in group 2 vs 1 with unchanged recovery of CO (*P*=NR), LVP (*P*=NS), dP/dt max (*P*=NR), and dP/dt min (*P*=NS) in group 4 vs 3
ATP and EC (ATP+0.5×ADP)/(ATP+ADP+AMP)[41]	DBD, preclinical (rat)	Parallel, 6-arm study with graft storage conditions, as described below, followed by 2 h normothermic MP: Groups 1–4: 200 min CSS (n=10) Groups 5–6: 200 min cold reperfusion (n=10)	Tissue samples after storage period	Myocardial function after 2 h normothermic MP	Not supported: ↑ ATP and EC (biomarkers) in groups 5–6 vs groups 1–4 with unchanged RPP

(*Continued*)

Table 5. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/ Predictive Value
EC (ATP+0.5×ADP)/(ATP+ADP+AMP), PCr×100/PCr+creatine and AMP/ATP ratio[32]	DCD, preclinical (pig)	Parallel, 2-arm study with 8–44 min WIT, followed by storage conditions described below, and 1 h unloaded, normothermic MP: 1. 4 h cold CSS (n=8) 2. 4 h cold reperfusion (n=8)	Tissue samples after 4 h storage	Myocardial function after 1 h normothermic MP	Supported: ↑ EC (P<0.01), PCr×100/PCr+creatine (P<0.05) and ↓ AMP/ATP (P<0.01) with ↑ heart rate (P<0.05), P_{max} (P<0.05), dP/dt max (P<0.05), RPP (P<0.01), and contractility index (P<0.05), ↓ dP/dt min (P<0.05) in group 2 (reperfusion) vs 1 (CSS)
				EC and AMP/ATP after 1 h normothermic MP	Supported: in parallel with biomarker changes (above), ↑ EC and ↓ AMP/ATP as outcomes (P<0.001 for both) in group 2 (reperfusion) vs 1 (CSS)
ATP[42]	DCD, preclinical (rat)	Parallel, 7-arm study (6 relevant arms) with 25 min global, warm ischemia, followed by storage conditions, as described below, and 1 h unloaded, normothermic MP: Group 1: 4 h CSS (n=14) Groups 2–6: 1 h reperfusion at 20°C, 25°C, 30°C, 33°C, or 37°C, respectively, and 4 h CCS (n=14)	Tissue samples after storage period	Myocardial function after 1 h normothermic MP	Supported: ↑ ATP (P<0.0001 for all) with ↑ heart rate (P<0.0001 for all) and mean dP/dt (P=0.0125 for all) in groups 2–6 vs 1
				ATP after 1 h normothermic MP	Not supported: in parallel with biomarker changes (above), unchanged ATP (P=NR) as outcome in groups 2–6 vs 1
ATP[43]	DCD, preclinical (rat)	Parallel, 3-arm study (2 relevant arms) with 25 min WIT followed by storage conditions, as described below, and 1 h, unloaded, normothermic MP: 1. 4 h CSS (n=11) 2. 1 h normothermic reperfusion and 4 h CSS (n=11)	Tissue samples after storage period	Myocardial function after 1 h normothermic MP	Mixed results Supported: ↑ ATP (biomarker; P<0.05) with ↑ heart rate (P=0.024) and mean dP/dt (P=0.042) in group 2 vs 1 Not supported: in parallel with biomarker changes (above), unchanged contractile index
				ATP after 1 h normothermic MP	Supported: in parallel with biomarker changes (above) ↑ ATP (outcome; P<0.05) in group 2 vs 1

CO indicates cardiac output; CSS, cold static storage; DBD, donation after brain death; DCD, donation after cardiac death; dP/dt, first derivative of left ventricular pressure; dP/dt max, maximal dP/dt; dP/dt min, minimal dP/dt; EC, energy charge; EGF, early graft failure; HEP, high-energy phosphate; HTx, heart transplantation; LVP, left ventricular pressure; LVSP, left ventricular systolic pressure; MP, machine perfusion; MRS, magnetic resonance spectroscopy; NR, not reported; NS, not significant; P_{max}, maximal developed pressure; PCr, creatine phosphate; Pi, inorganic phosphate; r, correlation coefficent for linear regression; R, Pearson correlation; RPP, rate-pressure product (heart rate×peak systolic pressure); and WIT, warm ischemia time.

patients have reported that donor plasma procalcitonin, when measured at the start of donor management or on pericardial opening, negatively correlates with cardiac graft function both before procurement and posttransplantation.[53–55] Furthermore, procalcitonin, measured immediately before pericardial opening, is an independent predictor of early graft failure in DBD.[54,55] Although procalcitonin may be elevated as a result of brain death, the precise stimulus and cell type responsible for its release are unknown.[56] As with BNP/NT-proBNP, the utility of procalcitonin, when measured at later stages of heart transplantation or in DCD heart transplantation, has not been investigated.

Copeptin

Copeptin may predict postoperative outcomes in heart transplant patients,[57] but to our knowledge, it has not been investigated in cardiac grafts. Copeptin is a portion of the prevasopressin-provasopressin molecule secreted by the hypothalamus and is emerging as a biomarker in various cardiac diseases, such as heart failure and acute coronary syndrome.[58] Copeptin is measurable in donor blood; however, it is a marker not only of cardiac damage, but also of pulmonary disease, diabetes mellitus insipidus, hemorrhagic shock, and ischemic stroke.[59] Thus, copeptin assessment could contribute information about cardiac cellular damage pre–heart transplantation, but likely requires interpretation in combination with more cardiac-specific biomarkers.

Cellular Damage/Death Markers

Although release of cellular damage/death markers can provide valuable information about cardiac injury, variable levels, which do not accurately reflect graft quality, may be present in donor blood as a result of previous defibrillation and in cardioplegia or MP perfusate solutions as a result of extended cold static storage or perioperative damage. These factors may explain the inconsistent findings for the utility of cardiac troponin T and cardiac troponin I as predictors for posttransplant graft function in clinical DBD studies (Table 6[17–19,54,60–66]). When investigated in this context, creatine kinase–muscle/brain isozyme levels at pericardial opening did not appear useful for human DCD graft evaluation.[63] Lack of predictive value may result from high interpatient variability for normal creatine kinase–muscle/brain isozyme values, as well as nonheart sources of circulating donor creatine kinase–muscle/brain isozyme (ie, skeletal muscle).[63] Lactate dehydrogenase was of some value in predicting functional recovery in a preclinical DCD model during MP[19]; however, as erythrocytes may also release lactate dehydrogenase, its utility with blood/erythrocyte-containing perfusates is likely limited. H-FABP (heart-type fatty acid binding protein) may also be a useful biomarker as it is rapidly released from cardiomyocytes following ischemic damage.[67,68] No study has addressed its potential as an indicator of cardiac damage in transplantation; however, it appears promising in acute myocardial infarction. Xu and colleagues reported a pooled sensitivity of 0.75 and a specificity of 0.81 for H-FABP alone in diagnosis of acute myocardial infarction within 6 hours.[67] The combination of H-FABP with high-sensitivity troponin T improved sensitivity, but reduced specificity.[67] H-FABP may be a particularly valuable indicator of graft damage as it can be detected earlier than troponins, from 0.5 to 1.5 hours,[68,69] versus 3 to 6 hours for cardiac troponin T in acute myocardial infarction.[70] Thus, the value of H-FABP in heart transplantation graft evaluation, especially during MP, is of particular interest for future investigation.

Inflammatory Markers

Endothelial Activation

Although preclinical data support the concept that endothelial activation is associated with reduced posttransplant outcomes, corroborative clinical studies are lacking (Table 7[25,71–76]). Stoica and colleagues demonstrated that endothelial activation (higher levels of P-selectin and vascular cell adhesion molecule 1) was increased in biopsies of DBD cardiac grafts compared with control tissue, but that changes in endothelial activation during transplantation are not predictive for organ failure.[71] Circulating endothelial microparticles, submicroscopic membrane vesicles released from the surface of endothelial cells during activation, injury, and/or apoptosis, are also of potential interest as biomarkers.[77] Although not yet investigated in the setting of graft evaluation, these particles indicate increased endothelial apoptosis in posttransplant patients.[77] Further investigation is required to elucidate the relationships between endothelial cell activation and microparticles, rejection, and cardiac allograft vasculopathy.

Inflammatory Cytokines

Inflammatory cytokines may be involved in graft dysfunction.[75,76] Reports investigating the utility of tumor necrosis factor (TNF)-α, soluble TNF receptors 1 and 2, and interleukin 6 (IL-6) as indicators of posttransplant graft function are presented in Table 7.

TNF-α protein levels can rapidly increase in cardiac myocytes after brain death and may have negative consequences, including activation of inducible

Table 6. Markers of Cell Death

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
cTnT[60]	DBD, clinical	Prospective, single-arm study (n=64)	Donor blood before pericardial opening	PGD	No (P=NR)	NR
cTnI[61]	DBD, clinical	Retrospective, exploratory study, 2 groups: 1. UW (n=23) 2. Custodiol HTK solution (n=20)	Preservation solution when donor heart removed from storage bag	PGD	Yes for UW (R^2=NR, P=0.031)	NR
					Yes for Custodiol HTK solution (R^2=NR, P=0.034)	
				Ischemic duration (time graft in storage bag)	Yes for UW (ρ=0.62, P=0.004)	
					No for Custodiol HTK solution (R=0.14, P=0.59)	
cTnI[62]	DBD, clinical	Retrospective meta-analysis with potential heart donors, 2 groups: 1. Elevated cTnI (n=98) 2. Normal cTnI (n=165) (used for HTx; n=139)	Donor blood at varying times during management	Post-HTx hospitalization	Supported, ↑ in group 1 vs 2 (P=0.044)	NR
				Survival after 30 d	Not supported (OR, 0.95; P=0.96)	
				Survival after 1 y	Not supported (OR, 0.81; P=0.73)	
				Early graft failure	Yes (OR, 68.4; P<0.0001)	
cTnT[54]	DBD, clinical	Retrospective study of multiorgan donors >10 y of age (n=92), 2 groups: 1. Good graft function after HTx (n=78) 2. Early graft failure after HTx (including intraoperative patient death attributable to myocardial failure, intra-aortic balloon pump use ≤12 h post-HTx, or LVEF <30% ≤12 h post-HTx; n=14)	Donor blood before pericardial opening	Early graft failure	Yes for cTnI >1.6 µg/L (OR, 42.7; P<0.0001)	No for procalcitonin (R=0.12, P=0.27)
cTnI and cTnT[63]	DBD, clinical	Retrospective study with multiorgan donors >10 y of age, 2 relevant groups: 1. Good graft function (n=68) 2. Impaired graft function (n=11)*	Donor blood before pericardial opening	Early graft failure	Yes for cTnI >1.6 µg/L, sensitivity of 73% and specificity of 94%	NR
					Yes for cTnT >0.1 µg/L, sensitivity of 64% and specificity of 98.5%	
				Acute graft failure	Yes for cTnI >1.6 µg/L (OR, 42.7; P<0.0001)	
					Yes for cTnT >0.1 µg/L (OR, 56.9; P<0.0001)	
				30 d mortality	Yes for cTnI >1.6 µg/L (OR, 6.8; P=0.006)	
					Yes for cTnT >0.1 µg/L (OR, 7.2; P<0.01)	
					Yes for cTnT >0.13 µg/L (OR, 22.4; P<0.005)	

(*Continued*)

Table 6. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
cTnT[64]	DBD, clinical	Prospective study (adults and children), 3 groups divided into patients who received hearts from donors with cTnT: 1. <1 ng/mL (n=6) 2. ≥1 ng/mL (n=8) 3. >5 ng/mL (n=2)	Donor blood during organ procurement	Severe ↓ in LVEFa	Yes (R=−0.59, P<0.0001)	NR
				Grade of rejection ≤1 y post-HTx	Yes (R=0.973, adjusted; R=0.943, P<0.001)	
cTnT[65]	DBD, clinical	Prospective study, 3 groups: 1. Normal donor LVEFa (≥50%; n=61) 2. Moderate decrease in donor LVEFa (30%–50%; n=25) 3. Severe decrease in donor LVEFa (≤30%; n=14)	Donor blood before HTx (exact time NR)	LVEFa in donor (simultaneous with biomarker)	Yes (ρ=−0.59, P<0.0001)	NR
cTnT[17]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C following WIT of: 1. 21 min 2. 24 min 3. 27 min 4. 30 min 5. 33 min (n=5–7 per group)	Perfusate samples at 10 min MP	LV work at 60 min MP	Yes (ρ=Nve, P<0.01)	NR
				TP at 60 min MP	Yes (ρ=Nve, P<0.05)	
				CO at 60 min MP	No (P=NR)	
				dP/dt max at 60 min MP	No (P=NR)	
				dP/dt min at 60 min MP	No (P=NR)	
cTnI[66]	DCD, preclinical (rat)	Randomized, prospective, parallel study with 4 relevant arms (n=75): 1. 0 min WIT 2. 5 min WIT 3. 10 min WIT 4. 20 min WITFollowed by MP, during which time hearts temporarily loaded mode for functional measurements	Right atrial plasma, immediately before heart procurement	Cardiac function during MP	Not supported: unchanged cTnI in parallel with ↓ ESPVR in groups 3 and 4 vs 1, ↓ dP/dt max in group 4 vs 1, and ↑ dP/dt min in groups 2–4 vs 1 (P<0.05 for all)	NR
			Coronary effluent at 15, 30, 45, 60 min MP	Cardiac function during MP†	Supported: ↑ cTnI in group 4 vs groups 1–3 at all sampling time points (P<0.05 for all) in parallel with functional changes (above)	
cTnT[18]	DCD, preclinical (rat)	Parallel, 3-arm study in hearts subjected to WIT, as described below, followed by 10 min procurement reperfusion, cardioplegic flush, CSS for 3 h 120 min loaded, normothermic MP: 1. 15 min WIT (n=6) 2. 20 min WIT (n=6) 3. 25 min WIT (n=5)	(A) At 10 min procurement reperfusion (before CSS) (B) At 10 min loaded, normothermic MP (after CSS)	Multiple functional parameters (CF, CO, PSP, DP, heart rate, dP/dt min, DP/dt max, RPP, TP) at 120 min loaded, normothermic MP	Yes for B: all outcomes (ρ=NR, P<0.05), except heart rate (P=NS)	NR
					No for A (P=NS for all)	

(*Continued*)

Table 6. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
CK-MB, CK-MB/CK, and myoglobin[63]	DBD, clinical	Retrospective study with multiorgan donors >10 y of age, 3 groups: 1. Good graft function (n=68) 2. Impaired graft function (n=11)* 3. Grafts not accepted for HTx (n=39)	Donor blood before pericardial opening	Not applicable	Not supported, no differences in CK-MB activity or CK-MB/CK ratio among groups	NR
CK-MB and CK-MB/CK[65]	DBD, clinical	Prospective study, 3 groups: 1. Normal donor LVEFa (≥50%; n=61) 2. Moderate decrease in donor LVEFa (30%–50%; n=25) 3. Severe decrease in donor LVEFa (≤30%; n=14)	Donor blood before HTx (exact time NR)	LVEFa in donor (simultaneous with biomarker)	Yes for CK-MB (ρ=−0.17, *P*=0.048) No for CK-MB/CK (*P*=NS)	NR
LDH[19]	DCD, preclinical (rat)	Parallel, 4-arm study, MP (20 min unloaded + 40 min loaded) at 37°C of hearts subjected to WIT of: 1. 30 min, 32°C (n=6) 2. 50 min, 32°C (n=5) 3. 55 min, 32°C (n=15) 4. 60 min, 32°C (n=5)	Perfusate samples, calculated as % change between 5 and 10 min MP	RPP, LV work at 60 min MP CO, DP, dP/dt max, dP/dt min, heart rate, PSP at 60 min MP	Yes (ρ=NR, *P*<0.05) No (*P*=NS)	NR

ρ indicates Spearman ρ; CF, coronary flow; CK, creatine kinase; CK-MB, CK–muscle/brain isozyme; CO, cardiac output; CSS, cold static storage; cTnI, cardiac troponin I; cTnT, cardiac troponin T; DBD, donation after brain death; DCD, donation after circulatory death; DP, developed pressure; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; ESPVR, end-systolic pressure-volume relationship; HTK, histidine-tryptophan-ketoglutarate; HTx, heart transplantation; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; LVEFa, LVEF area; LV work, left ventricular work (heart rate×DP); MP, machine perfusion; NR, not reported; NS, not significant; Nve, negative correlation; OR, odds ratio; PGD, primary graft dysfunction; PSP, peak systolic pressure; *R*, Pearson correlation; RPP, rate-pressure product (heart rate×PSP); TP, triple product (heart rate–DP–dP/dt max product); UW, University of Wisconsin preservation solution; and WIT, warm ischemia time.

*Impaired graft function defined as follows: intraoperative death caused by myocardial failure, intra-aortic balloon pump use for weaning from cardiopulmonary bypass or hemodynamic support ≤12 hours postoperatively, and LVEF <30% by echocardiography ≤12 hours postoperatively.

†Potentially simultaneous measurements of biomarker and outcome.

NO synthase, disruption of excitation-contraction coupling and systolic/diastolic function, inflammation, cardiac myocyte apoptosis, and even heart failure.[75] Although only few investigations of TNF-α in DBD heart transplantation have been reported, they consistently demonstrate elevated levels of TNF-α in cardiac tissue and donor serum samples for hearts with poor myocardial function, as well as for hearts that develop right heart failure early after transplantation.[75]

Interleukin 6 is released by immune cells, mesenchymal cells, endothelial cells, and fibroblasts, among others, into the circulation in response to various stimuli. Interleukin 6 has been investigated only in a few heart transplantation studies; nonetheless, levels in cardiac biopsies or donor blood negatively correlate with graft quality and patient outcomes.[73,75]

Importantly, as donor serum TNF-α levels correlate with tissue content in biopsies,[76] it may be that biopsy measurements could be avoided. Instead, inflammatory cytokine profiles of donor blood and/or MP perfusate could be evaluated, providing potential advantages for these biomarkers.

Damage-Associated Molecular Patterns

Tissue and cells undergoing stress or damage, such as ischemia-reperfusion injury, release molecules collectively termed damage-associated molecular patterns (DAMPs). DAMPs are analogous to pathogen-associated molecular patterns, with the activation of innate immunity, which may ultimately lead to graft inflammation and early graft dysfunction.[78] DAMPs include the following cellular and extracellular components: fibrinogen, nucleic acids (RNA and DNA), high-mobility group box 1, S100, HSPs (heat shock proteins) (HSP70/72, HSP90, and HSP60), tenascin c, and histone. Circulating levels of high-mobility group box 1, S100, HSP70, and tenascin c in patients with signs of acute ischemic coronary disease are potential biomarkers for early diagnosis of myocardial infarction.

Similarly, damaged mitochondria also liberate mitochondrial components of the DAMP class, termed mitochondrial DAMPs. Recognized mitochondrial DAMPs include the following: mitochondrial DNA, cytochrome c, ATP, mitochondrial transcription factor A, N-formyl peptides, succinate, and cardiolipin.[79] Importantly, increased circulating mitochondrial DAMPs in both DBD and DCD donors have been reported, and donor plasma mitochondrial DNA levels correlate with early allograft dysfunction in liver transplant recipients.[80] In an isolated rat heart model of DCD, we have recently demonstrated that cytochrome c and succinate are released during early reperfusion following warm, global ischemia, and levels negatively correlate with subsequently measured functional recovery.[17]

As DAMPs are ubiquitously expressed, evaluation of release during MP, rather than donor levels, is likely to provide superior specificity for cardiac graft evaluation.

New Approaches Toward Graft Evaluation

Omics Approaches

Investigation into patterns of changes in genomics (gene expression), transcriptomics (mRNA expression), proteomics (protein expression and posttranslational modifications), and metabolomics (metabolites) in cardiac grafts may also provide valuable information about graft quality. Indeed, mRNA signatures have been reported to detect injury both at procurement and during MP in a preclinical DCD model.[66] Furthermore, the development of pharmacogenomics approaches could potentially be used to individualize and optimize drug therapy during MP.[81]

Exosome Profiling

Exosomes, secreted nanovesicles of 50 to 200 nm with specific RNA, lipid, and protein content, are released into the circulation or body fluids in patients with various pathological conditions, indicating a potential role for exosomes as a diagnostic tool. Following heart transplantation, recipient dendritic cells acquire donor major histocompatibility complex molecules by capturing donor-derived exosomes,[82] implicating exosomes in the induction of antigen-specific effector immune responses.[67] This exosome-mediated major histocompatibility complex "cross-dressing" in immune homeostasis[83] may therefore be exploited as a biomarker to monitor acute rejection.[84]

Before transplantation, exosomes extracted from heart perfusate may reflect the pathophysiological status of the donor cells and organ. The number of donor exosomes has been demonstrated to correlate with organ stability/rejection in a mouse heart transplantation model.[69] Importantly, cardiac myosin and vimentin are recognized as tissue-restricted self-antigens that are detectable on the surface of circulating exosomes and are associated with primary graft dysfunction.[85] Even before cell membrane rupture, cardiomyocytes can release cardiac myosin and vimentin in association with exosomes. Not only are structural proteins enveloped in exosomes of damaged, but nondisrupted, cardiomyocytes, recently Hu et al showed that apoptosis-related proteins are enriched in exosomes released by cardiomyocytes subjected to oxidative stress.[86] Thus, it is plausible that following the catecholamine surge that accompanies donor death and/

Table 7. Endothelial Activation and Inflammatory Markers

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
P- and E-selectin, VCAM-1, thrombomodulin[71]	DBD +/– domino, clinical	Observational, parallel, 3-arm study: 1. Unused hearts (n=6) 2. Domino hearts (n=3) 3. DBD hearts (n=28)	Cardiac biopsies at multiple time points from initial donor assessment up to 3 mo posttransplant Time 1: at initial donor assessment Time 2: during CSS Time 3: at end of HTx Time 4: before release of cross-clamp Time 5: 10 min after reperfusion Time 6: 1 wk posttransplant Time 7: 1 mo posttransplant Time 8: 3 mo posttransplant	Allograft failure (time point not specified)	Not supported: for changes in biomarker expression (*P*=NS)	NR
ELP expression[72]	Heterotopic HTx, preclinical (mouse)	Parallel, 4-arm study: 1. WT donors and recipients (n=11) 2. $ELP^{-/-}$ donors in WT BALB/c recipients (n=6) 3. $ELP^{-/-}$ donors in bm12 recipients (n=10) 4. WT BALB/c donors in bm12 recipients (n=9)	Not measured, on the basis of transgenic model	Rejection score	Supported: ↓ rejection score in group 2 vs 1 (*P*<0.01)	NR
				Acute rejection (histologic analysis) at 3 d after HTx	Supported: ↓ mononuclear cell infiltration in group 2 vs 1 (*P*=NS)	
				Acute rejection (histologic analysis) at 5 d after HTx	Supported: ↓ mononuclear cell infiltration and coronary artery vasculitis in group 2 vs 1 (*P*<0.01)	
				Acute rejection (histologic analysis) at 8 d after HTx	Supported: ↓ necrosis in group 2 vs 1 (*P*=NR)	
				Chronic rejection at 8 wk	Supported: ↓ rejection score in group 3 vs 4 (*P*<0.01)	

(*Continued*)

Table 7. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
sTNFR1, sTNFR2, IL-6[73]	DBD, clinical	Prospective, single-arm study (n=43)	Donor blood at procurement	Recipient ICU stay	Supported: ↑ donor sTNFR1 when ICU stay ≤5 d vs >5 d (*P*=0.014)	NR
					Supported: ↑ donor sTNFR2 when ICU stay ≤5 d vs >5 d (*P*=0.03)	
				Recipient requirement for higher NOR doses after CPB weaning and during postoperative period	Supported: ↓ donor sTNFR2 when moderate/high doses of NOR required vs not required (*P*=0.028)	NR
					Supported: ↓ donor IL-6 when moderate/high doses of NOR required vs not required (*P*=0.001)	
				Recipient hospitalization time	Supported: ↑ donor IL-6 when hospitalization ≤25 d vs >25 d (*P*=0.029)	NR
IL-6, IL-6R, and gp130[74]	DBD, clinical	Parallel, 3-arm study with 2 relevant arms: 1. DBD donors (with good function: normal ejection fraction; n=6) 2. Controls (healthy patients; n=9)	Cardiac biopsies (immediately after heart procurement for DBD group)	On the basis of study groups	Supported: mRNA expression of all biomarkers for group 1 vs 2 (*P*<0.005)	NR
TNF-α[75]	DBD with or without domino HTx, clinical	Parallel, 2-arm study with DBD (n=16) and DBD domino (n=10) hearts	RV graft biopsies immediately before HTx	Recipients with (A) or without (B) right heart failure*	Supported: ↑ TNF-α mRNA expression in A vs B (*P*<0.005)	Correlations not statistically significant for: 1. Interferon-γ 2. IL-2 3. iNOS mRNA
					Supported: ↑ TNF-α protein expression in A vs B (*P*<0.05)	
					Supported: ↑ TNF-α expression in cardiac myocytes in A vs B (*P*=NS)	

(*Continued*)

Table 7. Continued

Biomarker	Model	Experimental Design	Biomarker Sampling	Outcome	Biomarker-Outcome Correlation/Predictive Value	Correlation With Other, Potential Predictive Marker
TNF-α and IL-6[76]	DBD, clinical	Parallel, 4-arm study with 2 relevant arms: 1. Unused donors (EF <30% coupled with 2 of: mean blood pressure <50 mm Hg, mean left atrial pressure >14 mm Hg, or inotropic support >0.4 μg×kg^{-1}×min^{-1}; n= 15) 2. Used donors (with cardiac function above criteria in group 2; n=31)	LV graft biopsies after CSS	On the basis of study groups	Supported for TNF-α mRNA, ↑ in 1 vs 2 (P<0.005)	NR
					Supported for IL-6 mRNA, ↑ in 1 vs 2 (P<0.0001)	
					Supported for TNF-α protein expression, ↑ in 1 vs 2 (P<0.05)	
					Supported for TNF-α in cardiac myocytes, ↑ in 1 vs 2 (P=NR)	
			Donor blood samples before procurement	On the basis of study groups	Supported for serum TNF-α, ↑ in 1 vs 2 (P<0.05)	
					Not supported for serum TNFR1, serum TNFR2, or serum IL-6, 1 vs 2 (P=NS for all)	
IL-10, IL-6, and TNF-α[25]	DCD, preclinical (rat)	Parallel, 5-arm study, MP (10 min unloaded+50 min loaded) at 37°C of hearts subjected to ischemia for: 1. 21 min (n=5) 2. 24 min (n=4) 3. 27 min (n=5) 4. 30 min (n=4) 5. 33 min (n=5)	IL-10 in cardiac tissue at 60 min MP	CO at 60 min MP	Yes (ρ=Nve, P<0.001)	Yes: -O_2C (ρ=Nve, P<0.05) -LDH (ρ=Pve, P<0.05) -Edema (ρ=Pve, P<0.05) -Peroxynitrite (100 kDa) (ρ=Pve, P<0.001) -Peroxynitrite (60 kDa) (ρ=Pve, P<0.05) -WIT (ρ=Pve, P<0.001)
				LV work at 60 min MP	Yes (ρ=Nve, P<0.05)	
				TP at 60 min MP	Yes (ρ=Nve, P<0.05)	
				DP at 60 min MP	No (P=NS)	
				dP/dt min at 60 min MP	No (P=NS)	
				dP/dt max at 60 min MP	No (P=NS)	
			IL-6 in cardiac tissue at 60 min MP	LV work at 60 min MP	No (P=NS)	Yes: -Peroxynitrite (100 kDa) (ρ=Pve, P<0.05) -Peroxynitrite (75 kDa) (ρ=Pve, P<0.05) -WIT (ρ=Pve, P<0.05)
				DP at 60 min MP	No (P=NS)	
				dP/dt min at 60 min MP	No (P=NS)	
				dP/dt max at 60 min MP	No (P=NS)	
				CO at 60 min MP	No (P=NS)	
				TP at 60 min MP	No (P=NS)	
			TNF-α in cardiac tissue at 60 min MP	LV work at 60 min MP	No (P=NS)	No
				DP at 60 min MP	No (P=NS)	
				dP/dt min at 60 min MP	No (P=NS)	
				dP/dt max at 60 min MP	No (P=NS)	
				CO at 60 min MP	No (P=NS)	
				TP at 60 min MP	No (P=NS)	

ρ indicates Spearman ρ; CO, cardiac output; CPB, cardiopulmonary bypass; CSS, cold static storage; DBD, donation after brain death; DCD, donation after circulatory death; DP, developed pressure; dP/dt max, maximal first derivative of left ventricular pressure; dP/dt min, minimal first derivative of left ventricular pressure; EF, ejection fraction; ELP, E-, P-, and L-selectin; gp130, glycoprotein 130; HTx, heart transplantation; ICU, intensive care unit; IL-2, interleukin 2; IL-6, interleukin 6; IL-6R, IL-6 receptor; IL-10, interleukin 10; iNOS, inducible NO synthase; LDH, lactate dehydrogenase; LV, left ventricle; LV work, left ventricular work (heart rate×DP); MP, machine perfusion; NOR, noradrenaline; NR, no reported; NS, not significant; Nve, negative correlation; O_2C, oxygen consumption; Pve, positive correlation; RV, right ventricle; sTNFR, soluble TNFR; TNF-α, tumor necrosis factor-α; TNFR, tumor necrosis factor receptor; TP, triple product (heart rate–DP–dP/dt max product); VCAM-1, vascular cell adhesion molecule 1; WIT, warm ischemia time; and WT, wild type.

*Right heart failure: dilated, poorly contracting RV, low CO, inotropic requirements >0.5 μg×kg^{-1}×min^{-1}, cardiac index <2, and metabolic acidosis.

or graft ischemia-reperfusion injury, damaged cells release exosomes into the circulation, which could lead to sensitization and eventually rejection in the recipient. Thus, exosomes may be of particular interest for graft evaluation not only posttransplantation to monitor rejection, but also in donor blood and/or in graft perfusate during MP.

Cardiac Imaging

Imaging techniques are already used to assess cardiac graft function in DBD donors; however, this may not be possible in all situations, particularly in DCD.

Echocardiography can assess myocardial function and anatomical structural abnormalities. With the Doppler technique to assess anatomic information, pressure gradients, and blood flow velocities, echocardiography can help in the diagnosis and grading of cardiac disease. Echocardiography is part of the standard donor heart evaluation procedure in DBD and is also used to assess DCD graft function during normothermic regional perfusion.[14] Despite strict limitations on antemortem interventions in DCD, Chew et al[10] reported the use of echocardiography to assess the function of potential donor hearts before withdrawal of life-sustaining therapy.

X-ray fluoroscopy is one of the most common examinations for imaging the coronary arteries. The utility of coronary angiography in donor heart assessment during MP has been demonstrated in 2 case reports.[87,88] Cardiac computed tomography is frequently used as a low-radiation alternative, for noninvasive, rapid screening for both coronary artery stenosis and structural abnormalities, such as valve disease.[89]

Nuclear imaging techniques have long been established, and new tracers are being developed constantly.[90] With radioactive tracers, various metabolic pathways can be investigated using single-photon emission computed tomography or positron emission tomography.[89] Disadvantaged by poor spatial resolution, fusion imaging methods, such as positron emission tomography with computed tomography or magnetic resonance imaging, improve accuracy of tracer localization and accumulation, and of tissue characteristics themselves.

Cardiovascular magnetic resonance is the gold standard for assessment of cardiac function, especially of the right ventricle, which is harder to assess with echocardiography as it does not follow a classic geometric model.[91] Cardiovascular magnetic resonance can be considered a comprehensive examination as it can also detect many factors, including, but not limited to, structural changes, such as fibrosis and scar, iron or fat accumulation, or other pathological conditions.[92] It also allows for the detection and (semi-) quantitative assessment of myocardial edema, which is of prognostic value in inflammatory diseases and ischemia.[93] The addition of contrast agents permits the detection of fibrosis and scar, as well as the assessment of coronary and microvascular function. More recent approaches focus on exploiting endogenous contrast and vasodilation techniques to assess microvascular function and myocardial oxygenation.[94,95] Furthermore, techniques, such as parametric mapping, permit quantification of tissue characteristics.[96] Mapping may be more sensitive to early-stage pathological conditions,[97] and reader bias is eliminated as evaluations are no longer based on visual assessment.

Magnetic resonance spectroscopy has mainly been used to assess energetic inorganic phosphates as the ATP/creatine phosphate ratio noninvasively in vivo,[98] but there are also various other molecules that can be traced, permitting measurement of even the deoxygenation of heme molecules.[99] It is ideally suited to studying cardiac metabolism; however, it is restricted to research and possesses limited sensitivity. Hyperpolarized metabolic magnetic resonance allows real-time metabolic imaging without the need for radioactive compounds[100]; however, it is in its infancy in humans.

In combination with imaging technologies, MP opens the door to new, ex situ possibilities, with the advantages of permitting imaging after the heart has already been exposed to potentially damaging conditions, such as warm ischemia and reperfusion in DCD, and the ability to monitor multiple variables rapidly. Nonetheless, the use of contrast agents must be carefully considered, and purpose-built perfusion systems may be required.

CONCLUSIONS

Major obstacles in heart transplantation include insufficient donor organs and high rates of primary graft dysfunction. Improved protocols for cardiac graft evaluation may not only aid in expanding the donor pool, but may also provide better clinical outcomes by helping to identify grafts at risk for primary dysfunction. A greater consideration of cardiac biomarkers, be they measured at procurement, during static storage, and/or during MP, holds potential for developing clinical protocols that allow a more precise and reliable evaluation of graft suitability for transplantation. This is of particular interest, on one hand, in light of increasing use of ECDs and DCD, which may require more sophisticated graft evaluation techniques; and, on the other hand, as cardiac MP options, which enable monitoring the profile of multiple, graft-specific parameters, become available.

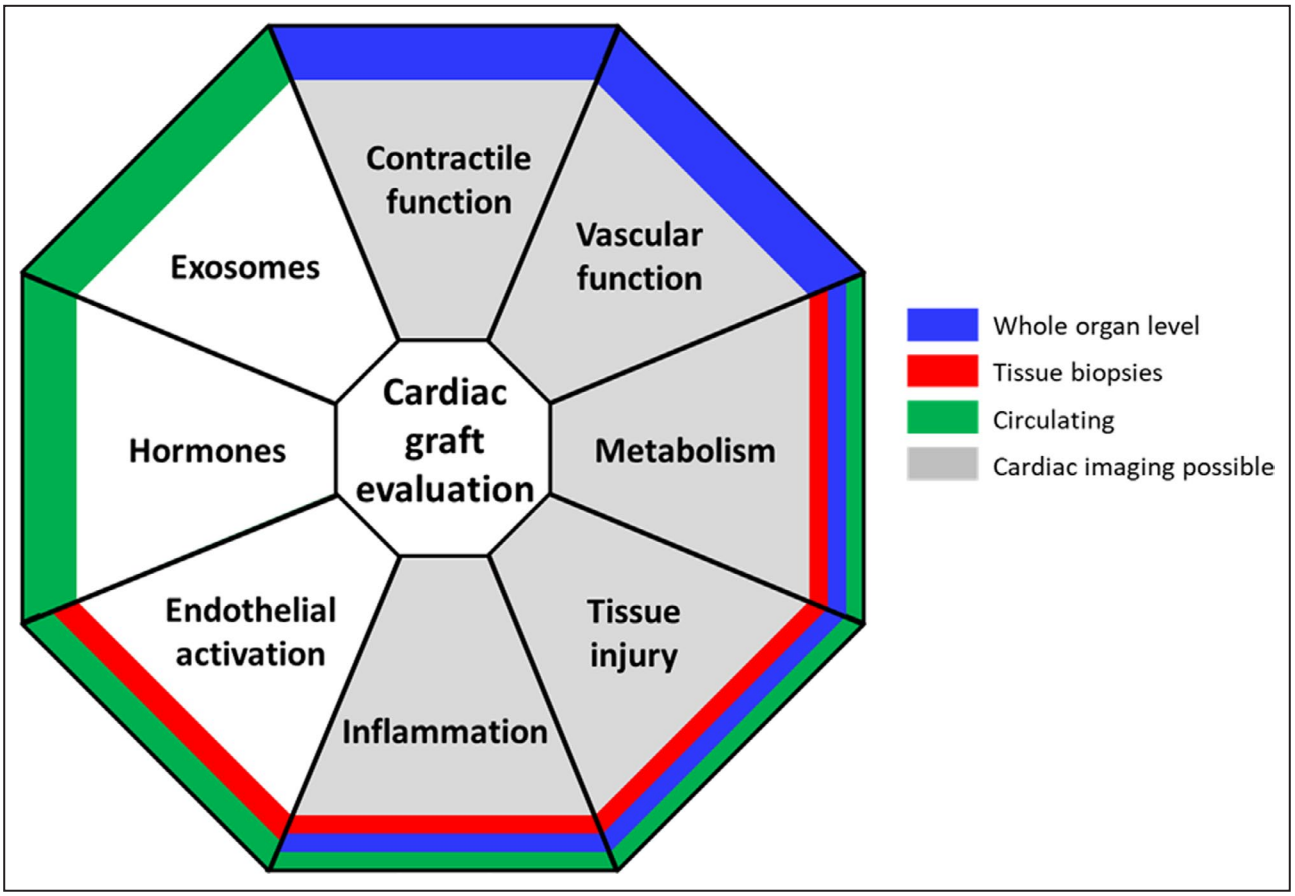

Figure 3. Schematic overview of markers for cardiac graft evaluation.
Markers can be assessed at the level of the whole organ (blue), tissue biopsies (red), or circulating in donor blood or ex situ perfusate (green). Cardiac metabolism: cardiac oxygen consumption and efficiency, lactate release, and high-energy phosphate metabolites. Tissue injury: cardiac troponin I and cardiac troponin T. Endothelial activation: P-, E-, and L-selectin expression, vascular cell adhesion molecule-1, and thrombomodulin. Vascular function: coronary flow, vascular relaxation, vascular leakage (edema), and endothelial NO synthase coupling (peroxynitrite formation). Inflammation: tumor necrosis factor (TNF)-α, interleukin 6, interleukin 10, glycoprotein 130, and soluble TNF receptors 1/2. Hormones: procalcitonin and brain natriuretic peptide.

In this review, we have identified and summarized particularly promising approaches in the development of biomarkers for cardiac graft quality and provided indications for the time window of assessment (Figure 3). Importantly, MP allows monitoring of changes in biomarker release over time via perfusate sampling. This possibility should permit more precise graft evaluation. Nonetheless, identification of reliable predictive parameters will require standardization of perfusion conditions, such as perfusion temperature, composition, and pressure, as well as chronotropy and inotropy. Furthermore, techniques must permit timely biomarker measure evaluation to fit within the time frame of clinical heart transplantation protocols. Several biomarkers, such as troponins and lactate, are rapidly and routinely measured in clinical practice and could be implemented without difficulty. However, other approaches, such as HEP or exosome profiling, which may be of particular value in graft evaluation, require further development to establish rapid measurement protocols and appropriate thresholds/criteria. Importantly, the combined use of several biomarker measurements would likely provide the most robust assessment. Current imaging modalities could also be used to evaluate cardiac grafts, permitting detailed graft characterization. Of particular relevance are techniques that enable the assessment of metabolic flux and allow optimization of the perfusion environment with a high potential for translatability and graft assessment before transplant without the need for radioactive tracers. Although MP systems must be specially adapted for computed tomography and cardiovascular magnetic resonance, imaging modalities are particularly attractive as several graft characteristics can be simultaneously evaluated. Cardiovascular magnetic resonance is of particular promise as it possesses excellent spatial and temporal resolution, it is not limited by poor ultrasound windows, as may occur with echocardiography, and does not use ionizing radiation.

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Sources of Funding

None.

Disclosures

None.

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